IN THE CLAIMS

The claims are as follows:

1-34. (Canceled)

35. (Withdrawn, Previously Presented) A method to detect or determine the presence or amount of a mutant hydrolase, comprising:

a) contacting a mutant hydrolase with a hydrolase substrate which comprises one or more functional groups, wherein the mutant hydrolase comprises at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the at least one amino acid substitution results in the mutant hydrolase forming a bond with the substrate which is more stable than the bond formed between the corresponding wild-type hydrolase and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate, wherein the wild-type hydrolase is a dehalogenase, wherein the mutant hydrolase is a mutant dehalogenase, and wherein the substrate is a compound of formula (I): linker R-linker-A-X, wherein the linker is a branched or unbranched carbon chain comprising from 2 to 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A-X is a substrate for a dehalogenase, wherein A is $(CH_2)_n$ and n = 2-10, wherein X is a halogen, and wherein R is a biotin functional group coupled through its carboxy terminus to the linker; and

b) detecting or determining the presence or amount of the functional group, thereby detecting or determining the presence or amount of the mutant dehalogenase.

36. (Withdrawn, Previously Presented) The method of claim 35 wherein the substitution is at a residue in the wild-type dehalogenase that activates the water molecule.

37. (Withdrawn, Previously Presented) The method of claim 36 wherein the residue in the wild-type dehalogenase that activates the water molecule is histidine.

38. (Withdrawn, Previously Presented) The method of claim 35 wherein the substitution is at a residue in the wild-type dehalogenase that forms an ester intermediate with the substrate.

39. (Withdrawn, Previously Presented) The method of claim 38 wherein the residue in the wild-type dehalogenase that forms an ester intermediate with the substrate is aspartate.

40. (Withdrawn, Previously Presented) A method to isolate a molecule, cell or subcellular organelle of interest in a sample, comprising:

contacting a sample suspected of having a molecule, cell or organelle of interest with a fusion protein comprising a mutant hydrolase and a protein which binds the molecule, cell or subcellular organelle of interest, and a hydrolase substrate which comprises one or more functional groups, wherein the mutant hydrolase comprises at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the at least one amino acid substitution results in the mutant hydrolase forming a bond with the substrate which is more stable than the bond formed between the corresponding wild-type hydrolase and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves the bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate, wherein the wild-type hydrolase is a dehalogenase, wherein the mutant hydrolase is a mutant dehalogenase, and wherein the substrate is a compound of formula (I): R-linker-A-X, wherein the linker is a branched or unbranched carbon chain

comprising from 2 to 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A-X is a substrate for a dehalogenase, wherein A is $(CH_2)_n$ and n = 2-10, wherein X is a halogen, and wherein R is a biotin functional group coupled through its carboxy terminus to the linker;
wherein if the sample comprises the molecule, cell or subcellular organelle of interest, the molecule, cell, or subcellular organelle is isolated from the sample by the formation of a complex between the molecule, cell, or subcellular organelle, the fusion protein, and the hydrolase substrate.

41. (Withdrawn, Previously Presented) The method of claim 40 wherein the substitution is at a residue in the wild-type dehalogenase that activates the water molecule.

42. (Withdrawn, Previously Presented) The method of claim 41 wherein the residue in the wild-type dehalogenase that activates the water molecule is histidine.

43. (Withdrawn, Previously Presented) The method of claim 40 wherein the substitution is at a residue in the wild-type dehalogenase that forms an ester intermediate with the substrate.

44. (Withdrawn, Previously Presented) The method of claim 43 wherein the residue in the wild-type dehalogenase that forms an ester intermediate with the substrate is aspartate.

45. (Canceled)

46. (Withdrawn) The method of claim 40 wherein the molecule of interest is a protein.

47. (Withdrawn, Previously Presented) A method to label a cell, comprising:

a) contacting a cell comprising a mutant hydrolase with a hydrolase substrate which comprises one or more functional groups, wherein the mutant hydrolase comprises at least one amino acid substitution relative to a corresponding wild-type hydrolase, wherein the at least one amino acid substitution results in the mutant hydrolase forming a bond with the substrate which is more stable than the bond formed between the corresponding wild-type hydrolase and the substrate, wherein the at least one amino acid substitution in the mutant hydrolase is a substitution at an amino acid residue in the corresponding wild-type hydrolase that is associated with activating a water molecule which cleaves a bond formed between the corresponding wild-type hydrolase and the substrate or at an amino acid residue in the corresponding wild-type hydrolase that forms an ester intermediate with the substrate, wherein the wild-type hydrolase is a dehalogenase, wherein the mutant hydrolase is a mutant dehalogenase, and wherein the substrate is a compound of formula (I): R-linker-A-X, wherein the linker is a branched or unbranched carbon chain comprising from 2 to 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A-X is a substrate for a dehalogenase, wherein A is $(CH_2)_n$ and n = 2-10, wherein X is a halogen, and wherein R is a biotin functional group coupled through its carboxy terminus to the linker; and

b) detecting or determining the presence or amount of the functional group.

48. (Withdrawn, Previously Presented) The method of claim 47 wherein the substitution is at a residue in the wild-type dehalogenase that activates the water molecule.

49. (Withdrawn, Previously Presented) The method of claim 48 wherein the residue in the wild-type dehalogenase that activates the water molecule is histidine.

50. (Withdrawn, Previously Presented) The method of claim 47 wherein the substitution is at a residue in the wild-type dehalogenase that forms an ester intermediate with the substrate.

51. (Withdrawn, Previously Presented) The method of claim 50 wherein the residue in the wild-type dehalogenase that forms an ester intermediate with the substrate is aspartate.

52-54. (Canceled)

55. (Withdrawn, Previously Presented) The method of claim 35, 40 or 47 wherein the linker comprises $(CH_2CH_2)_y$ and y = 2-8.

56. (Withdrawn, Previously Presented) The method of claim 35, 40 or 47 wherein the linker separates R and A by at least 12 atoms.

57. (Canceled)

58. (Withdrawn, Previously Presented) The method of any one of claims 35, 40 or 47 wherein the mutant dehalogenase is present in a cell or on the surface of a cell.

59-63. (Canceled)

64. (Withdrawn, Previously Presented) The method of any one of claims 35, 40 or 47 wherein the presence of at least one functional group in a cell is correlated to the subcellular location of the mutant dehalogenase.

65. (Withdrawn, Previously Presented) The method of any one of claims 35 or 47 wherein the mutant dehalogenase forms a fusion protein with a protein of interest.

66. (Withdrawn) The method of claim 65 wherein the protein of interest is a selectable marker protein, membrane protein, cytosolic protein, nuclear protein, structural protein, an enzyme, an enzyme substrate, a receptor protein, a transporter protein, a transcription factor, a channel protein, a phospho-protein, a kinase, a signaling protein, a metabolic protein, a mitochondrial protein, a receptor associated protein, a nucleic acid binding protein, an extracellular matrix protein, a secreted protein, a receptor ligand, a serum protein, an immunogenic protein, a fluorescent protein, or a protein with reactive cysteine.

67. (Withdrawn, Previously Presented) The method of claim 47 wherein the mutant dehalogenase further comprises a selectable marker protein.

68. (Canceled)

69. (Withdrawn, Previously Presented) The method of claim 67 wherein the mutant dehalogenase forms an ester bond with the substrate.

70. (Withdrawn, Previously Presented) The method of claim 67 wherein the mutant dehalogenase forms a thioester bond with the substrate.

71. (Withdrawn) The method of claim 47 further comprising contacting the cell with a fixative prior to or after contacting the cell with the substrate.

72. (Withdrawn) The method of claim 47 further comprising contacting the cell with a fixative concurrently with contacting the cell with the substrate.

73. (Withdrawn) The method of claim 71 or 72 wherein the cell is fixed with methanol, acetone and/or paraformaldehyde.

74. (Withdrawn) The method of claim 67 further comprising contacting the cell with a fixative prior to or after contacting the cell with the substrate.

75. (Withdrawn) The method of claim 67 further comprising contacting the cell with a fixative concurrently contacting the cell with the substrate.

76. (Withdrawn) The method of claim 74 or 75 wherein the cell is fixed with methanol, acetone and/or paraformaldehyde.

77. (Withdrawn) The method of claim 52 wherein the mutant dehalogenase is encoded by a nucleic acid sequence which is optimized for expression in a selected host cell.

78-106. (Canceled)

107. (Previously Presented) A method for preparing a compound of formula R-Linker-A-X comprising coupling a compound of formula R-Y with a compound of formula Z-Linker-A-X, wherein Y and Z are groups that can react to link R- to –Linker-A-X, wherein the linker is a branched or unbranched carbon chain comprising from 2 to 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A-X is a substrate for a dehalogenase, wherein A is $(CH_2)_n$ and n = 2-10, wherein X is a halogen, wherein R is a biotin functional group that is capable of being coupled through its carboxy terminus to the linker, and wherein R-Y is an activated ester of a compound of formula R and wherein Z is an amine suitable to react with the activated ester to form an amide bond.

108. (Canceled)

109. (Previously Presented) A method for preparing a compound of formula R-Linker-A-X wherein the Linker comprises an amide bond comprising coupling a corresponding activated ester with a corresponding amine to provide the compound of formula R–Linker-A-X, wherein R is a biotin functional group, wherein the linker is a branched or unbranched carbon chain comprising from 2 to 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein A-X is a substrate for a dehalogenase, wherein A is $(CH_2)_n$ and n = 2-10, and wherein X is a halogen.

110. (Previously Presented) A compound of formula (I): R-linker-A-X, wherein R is one or more functional groups, wherein the linker is a branched or unbranched carbon chain comprising from 2 to 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A is $(CH_2)_n$ and n = 4-10, wherein A-X is a substrate for a dehalogenase, and wherein X is a halogen, wherein R is a biotin functional group coupled through its carboxy terminus to the linker.

111. (Previously Presented) The compound of claim 110 which is a substrate for a *Rhodococcus* dehalogenase.

112. (Previously Presented) The compound of claim 110 wherein X is Cl or Br.

113. (Previously Presented) The compound of claim 110 wherein the linker comprises 3 to 30 atoms.

114. (Previously Presented) The compound of claim 110 wherein the linker has 11 to 30 atoms.

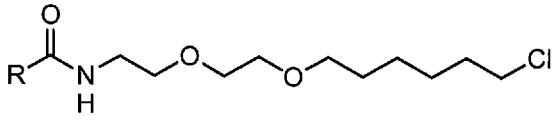
115. (Previously Presented) The compound of claim 110 which is N-{2-[2-(6-Chlorohexyloxy)-ethoxy]-ethyl}-biotin-amide.

116. (Previously Presented) The compound of claim 110 wherein R is separated from A-X by up to 100 angstroms.

117. (Previously Presented) The compound of claim 110 wherein R is separated from A-X by up to 500 angstroms.

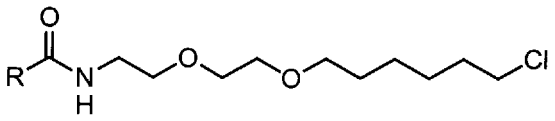
118. (Previously Presented) The compound of claim 110 wherein the chain comprises $(CH_2CH_2O)_y$ and y = 2-8.

119. (Previously Presented) A compound prepared by the method of claim 107 wherein the compound is



120. (Previously Presented) A compound of formula (I): R-linker-A-X, wherein R is one or more functional groups, wherein the linker is a branched or unbranched carbon chain comprising from 2 to 30 carbon atoms, which chain optionally includes one or more double or triple bonds, and which chain is optionally substituted with one or more hydroxy or oxo (=O) groups, wherein one or more of the carbon atoms in the chain is optionally replaced with a non-peroxide -O-, -S- or -NH-, wherein the linker-A separates R and X by at least 11 atoms, wherein A is $(CH_2)_n$ and n = 2-10, wherein A-X is a substrate for a dehalogenase, wherein X is a halogen, and wherein R is a biotin functional group coupled through its carboxy terminus to the linker.

121. (Previously Presented) A compound of formula (II):



122. (Previously Presented) A compound prepared by the method of claim 107.